SYNOPSIS

STUDIES ON BIOLOGY, HOST RANGE AND DESIGN STRATEGIES TO SUPPRESS EARLY DEVELOPMENTAL STAGES OF *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE)

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Introduction:

Bruchids are recognized as the most detrimental storage pest of pulses especially in the tropics and subtropics. They invade mature pods as well as seeds during storage. In India, approximately 200 species of insects have been recorded infesting various pulses. Among the storage pests, bruchids have greater importance specially *Callosobruchus maculatus* (Fab.) is the major pest causing serious damage and are cosmopolitan in distribution. *Callosobruchus* spp., though attack these crops in field and are carried to store where they continue to damage the grains (Singh and Ahlawat, 2005). Insects cause losses to stored grains throughout the world and their impact are more devastating in developing countries (Ekeh *et al.*, 2013). Both the crops in fields and the grains in stores are infested by a large number of insect pests.

Pulses are the second most important group of crops worldwide and have played a vital role in the improvement of agricultural economy of different countries (Deeba *et al.*, 2006). Pulses are drought resistant, suitable for dry land farming and predominantly used as intercrop with other crops. Pulses are main sources of proteins (20-40%), carbohydrates (50-60%) and are also good sources of thiamin, niacin, calcium and iron. pulses provide a perfect cocktail of essential amino acids with high biological value when supplemented with cereals. Therefore, pulses are considered as the primary protein source for vegetarians and are also called the poor man's meat (Reddy, 2010). This is named so because most of the poorer sections of the population are not able to afford non-vegetarian items for their diet. In fact, pulses contribute a lot for them as they get vegetable proteins, along with minerals and vitamins in their diet, while investing less. Nutritional value of pulse includes dietary fibres, complex carbohydrates, resistant starch, vitamin complex and minerals (Patterson *et al.*, 2009).

Pulses are an important group of food crops that can play a vital role to address national food and nutritional security and also tackle environmental challenges. The share of pulses to total food grain basket is around 9-10 per cent and are a critical and inexpensive source of plant-based proteins, vitamins and minerals.

In India, pulses are generally produced in poor soils not suited to other crops, with a minimum use of resources and have a very low water footprint. They are vital constituent of cropping and consumption pattern. Out of the total net sown area of 141.40 million hectares, 52 per cent i.e. 73.20 million hectares is rainfed. The pulse cultivation occupies major area under this ecology. The rainfed regions of the country support 40 per cent of human population and 2/3rd of livestock. As high as 90% of nutri-cereals (millets), 80% pulses, 74% oilseeds, 65% cotton and 48% rice crops are rainfed.

The total world area under pulses is about 85.40 (Mha) with production of 87.40 (Mt) at 1023 kg/ha yields level. India, with >29 Mha pulses cultivation area, is the largest pulse producing country in the world. It ranks first in area and production with 34 per cent and 26 per cent respectively. During 2017-18 the country's productivity at 835 kg/ha, is a significant increase over Eleventh (662 kg/ha) and Twelfth plans (745 kg/ha) (Pulses Revolution, Food to Nutritional Security, Min. of Agri. & FW (DAC&FW), GOI).

One of the major restrictions in the production of pulses is the attack of insect pests, which inflict severe losses both in the field and stores (Sherma, 1989). *Callosobruchus maculatus* causes serious threat to various pulses. These are known to feed and breed on different pulses. Knowledge of the host range and biology of the pest species are essential to minimize threat. Different control measures include chemical, physical, biological and cultural employed to combat these devastating pests for seed production and storage. Losses attributed to bruchids are estimated to be about 87 to 100% within storage duration of 3 to 6 months. It results in both quantitative and qualitative reduction of food and nutrition insecurity. It may also results in low incomes to small scale farmers (Ojiako *et al.*, 2013). Consequently, there is a need for new approaches especially botanicals to control insect pests in order to enhance food security among the people.

Literature Review:

Storage pests that feed on seeds have always been one of the main biotic constraints for pulse crops, and cause considerable economic losses during the post-harvest storage period. Among storage pests, the bruchids have adversely affected and greatly reduced the economic importance of the pulses (Srivastava and Pant, 1989; Ramzan *et al*, 1990). The damage caused by the bruchids during storage, is varies from crop to crop and also depend upon the species of bruchid and its biotype. For example, *Callosobruchus maculatus* causes up to 90% yield loss in black gram (Soundrarajan and Chitra, 2012), and 4–90% in cowpea (Maina *et al.*, 2012; Amusa *et al.*, 2013), depending upon the cultivar and their seed composition (Shivanna *et al.*, 2011; Radha & Susheela, 2014)

The cowpea bruchid, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), widely distributed in the tropical and subtropical regions is the most destructive pest of cowpea during storage (Deshpande *et al.*, 2011). *C. maculatus* attack cowpea in the field and storage conditions. It cause up to 44.7% depletion in weight, loss in the germination ability and the market and nutritional values of cowpea seeds (Oluwafemi, 2012; NARO, 2012; Adekunle and Ayodele, 2014; Miesho *et al*., 2018). Postharvest loss to storage coleopterans pests such as cowpea bruchid, *C. maculatus* is a major constraint to food security in developing nations such as Nigeria (Markham *et al.*, 1994; Udo, 2011).

Insect pest control is solely relied on synthetic chemical control, which has environmental health hazards to seed handlers and other non-targeted organisms (Gbaye and Holloway, 2011; Udo, 2011; Ileke *et al.*, 2016). Research effort on the use of botanicals in the control of coleopteran pests has been intensified by many researchers (Adedire and Lajide, 1999; Adedire and Akinkurolere, 2005; Adedire *et al.*, 2011, Akinkurolere *et al.*, 2006; Ileke and Olotuah, 2012; Ileke *et al.*, 2012, 2016).

Most of the damage caused by insects to grain is done in stored condition. Storage pest damage to grains reduce the grain weight (dry matter reduction), makes them unsuitable for human and animal consumption, food contamination with live or dead insects and depreciation of the nutritional and commercial values of the infested product and cause poor germination ability (Elhag, 2000). Grain insect pests may be divided into primary and

secondary pests. Primary grain insects have the ability to attack whole and most of the time on unbroken grains, while secondary pests attack on damaged grain and milled products. Heavy grains infestation can lead to mouldiness thereby causing a reduction in commercial value of the grains (Kiradoo and Srivastava, 2010).

Bruchids breed rapidly under storage conditions and by the time they are detected, the infested grain is usually unmarketable. Even an extremely low bruchid population could result in major damage within 4-6 months. Bruchids include various insect pests which damage different type of pulses in storage condition; these are *Acanthoscelides obtectus, Bruchus pisorum, Callosobruchus analis, Callosobruchus chinensis, Callosobruchus maculatus,* etc. It is very common that *Callosobruchus* sp. cause infestation to pulse crops in field. (Raina, 1970; Mohan and Subbarao, 2000). *Callosobruchus* sp. cause major damage to different pulses. The genus *Callosobruchus* attacks grain legumes during both pre and post-harvest stages all over world; but in India, *Callosobruchus maculatus, Callosobruchus analis, Callosobruchus chinensis* are pre-dominant pest species (Jat *et al*., 2013). *Callosobruchus maculatus* a cosmopolitan bruchid, is a continuous pest from the field to store (Jiao *et al*., 2011). It starts infestation in the field but heavy damage is done after storage (Swell and Mushobozy, 2007).

Callosobruchus maculatus is commonly known as cowpea weevil or cowpea seed beetle. The cowpea weevil lacks the "snout" as compared to true weevil. The cowpea weevil, *Callosobruchus maculatus,* is a major pest of economically important leguminous grains, such as cowpeas, lentils, green gram, soybean, black gram, etc. (Mulatu and Gebremedhin 2000, Raja *et al*., 2000, Park *et al*., 2003). The damage caused by this insect pest is mostly due to its larval feeding on the nutritional contents inside the grains which leads to weight loss, inferior quality and quantity properties, market value, as well as the germination potential of the infested grains. The extent of weight loss due to the damage of pulse beetle is quite variable and depends upon the storage period, storage conditions, storage containers and varieties of legume grains (Nchimbi-Msolla and Misangu, 2002).

Out of total pulse production in India, about 8.5% is lost during post harvest handling and storage. Thus, if bruchid pest infestation is taken into consideration, a comprehensive relationship will be established between the commodities stored, their

relative production and product loss during storage i.e. the quantity of pulses produced is directly proportional to the product to be stored and losses during storage. Therefore, Securing the harvest against storage pests is necessary because production of most food crops is done only once in a season under a rain-fed system.

The chemical pesticides were used in past to control these pests but their indiscriminate use caused emergence of resistance strains, pests resurgence, pollution etc. The traditional knowledge reveled that farmers use the plant parts and their derivatives as alternative insecticides in protecting food grains (Isman, 2000). Over the last 15 years, interest in botanical insecticides has increased as a result of environmental concerns and insects population become resistant to conventional chemical pesticides.

Synthetic insecticides have played a historical role in agriculture development in general and particularly in the postharvest grain storage (Ogendo and Hagstrum, 2004). However, their use by farmers is criticized worldwide (Ngowi *et al.*, 2005); although the importance of insecticides in storage pest control has been clearly established. But the misuse of chemicals causes negative effects to the human health and environment (Williamson *et al*., 2016).

The insecticidal activity of essential oils and plant extracts has been evaluated and found satisfactory for various stored grains and agriculture insect pests. The use of plant material for grain preservation and particularly cowpea is considered as a promising alternative to synthetic insecticides for several decades. Plant material may produce volatile chemicals that repel or confuse the adult beetles, eventually preventing invasion or causing emigration from treated stocks (Boeke *et al.,* 2004). These are also safer to human health and environment (Rehman *et al.,* 1992).

The ecological consequences and the increase of insect resistance to pesticides have led to a search for new classes of insecticides with lower mammalian toxicity and a lower persistence in the environment (Roger & Hamraoui, 1994). Sighamony *et al.,* (1984) tested oils of clove, cedar wood (*Juniperus virginiana*), karanja (*Pongamia glabra*) and an acetone extract of black pepper (*Piper nigrum*) to determine their repellent effects on adults of *Tribolium castaneum*. Cedar wood, karanja and pepper products were found to be more potent than the standard repellent, dimethylphthalate. Miah *et al.* (1993) reported the effects

of several Bangladeshi plant materials against *C. chinensis* on chickpea seeds. Nishinda (*Vitex engundo*) leaf powder was the most effective in reducing numbers of eggs laid, adult emergence and seed weight loss.

The different spices such as flower of clover, rhizomes of ginger and turmeric, fruits of black pepper, chilli pepper and bulb of garlic were used against *C. maculatus*. In this study, it was reported that cloves and black pepper gave significant result in terms of oviposition and seed weight, as compared to other spices used (Iqbal and Poswal, 1995).

Aslam *et al.,* (2002) tested six spice powders against *C. chinensis*. Clove and black pepper found good protectants of stored chickpea against the beetle. Kim *et al.,* (2003) showed the strong insecticidal activity of extract from cinnamon (*Cinnamomum cassia*) bark and oil, horseradish (*Cocholeria aroracia*) oil, and mustard (*Brassica juncea*) oil against *C. chinensis*. Asif (2007) tested plant powders and extracts of black pepper, red chillies, cloves, neem, datura (*Datura stramonium*), garlic and turmeric against pulse beetles and found the neem extract to be most insecticidal.

Essential oils found from *Cinnamomum zeylanicum* fruit and *Micromelum minutum* leaves. These were put to test for potential insecticidal activity against *C. maculatus*. The adults of *C. maculatus* were susceptible to both fumigant and contact toxicity of *C. zeylanicum* fruit oil and *M. minutum* leaf oil (Paranagama and Gunasekera, 2011).

A number of medicinal plants and spices have been used as pest control agents (Ani, 2010; Yankanchi and Gonugade, 2009). Researchers claim the successful use of vegetable oils (Kalita *et al*., 2014), plant extracts (Chiasson *et al*., 2004; Devanand and Rani, 2008; Yankanchi, 2009), and botanical powders (Patil *et al*., 2006; Shukla *et al*., 2007; Gupta and Srivastav, 2008., Kamran *et al*., 2015).

India grows a variety of pulse crops such as Bengal gram, red gram, green gram, black gram, dry peas, lentils, etc. under a wide range of agro-climatic conditions (Avinash and Patil, 2018). The increasing population, production shortages and consequent price rise of pulses have substantially reduced the per capita consumption of pulses across the world, and particularly in India, that is from 63.0 g day−1 in 1961 to 27.3 g day−1 in 2010 (Kumar *et al.,* 2011). Even this reduction trend in per capita consumption is expected to go down

further in future on account of less productivity due to climatic changes, the diversion of cultivated lands for urbanization and alterations in food habits. Several approaches including cultural, biological, physical and chemical control measures have been implemented with the aim of managing these pests, but none of these have been successful across time and space.

Research Gap Identification:

Legumes (pulses) provide high quality protein and considered to be the best food for vegetarian population in developing countries. The grain legumes are, however, susceptible to different species of beetles of the family bruchidae in storage commonly known as Dhora. At present insect management procedures frequently rely on synthetic insecticides and fumigants. Insecticides have wide and unsystematic application which cause natural imbalance and outbreak of secondary insects thus creating phyto-toxicity. Furthermore, non-stop use of insecticides has severe ill effects on insect pollination, biological agent like different predators, parasitoids and also become cause of the ecological contamination. Because of these drawbacks, internationally scientists are annoying to approve substitute methods of insect manage. There are different plant species, spices, medicinal plants used as powders, extracts and essential oils to control the *Callosobruchus maculatus*. But no/less study on powders of different plant of Himachal Pradesh has been carried out till date.

Proposed Methodology:

Sample Collection and Maintenance of stock culture: Seeds of different pulses shall be collected from grocery shop and kept in air tight containers. Pulse beetles, *Callosobruchus maculatus* shall be collected from old stored grains already infested with the pest and shall be taxonomically validated with the help of some Govt. agency. Pulse beetle shall be reared in the laboratory at ambient room temperature (27.6±2.5ºC) in glass jars.

Approximately, 100 adults of the collected pulse beetles shall be released in each jar containing 250 g of pulse seeds and the mouth shall closed with a piece of nylon cloth. The beetles shall be allowed for free mating and oviposition. Then the beetles shall be separated from the seeds by sieving and seeds along with eggs shall be kept in separate container for emergence of next generation. After emergence, the newly emerged adults shall be collected and again allowed for further mating and oviposition with new seeds in different containers to maintain a series of stock culture of the test insect. *Callosobruchus maculatus* shall be cultured on different pulses in different petri dishes.

Developmental Biology& Host Range: The life cycle and developmental stages of *Callosobruchus maculatus* shall be observed on different stored pulses viz. *Vigna ungiculata* (cowpea), *Vigna radita* (green gram), *Vigna mungo* (black gram) *and Glycine max* (soybean) etc. by introducing one pair of freshly emerged male and female in each petri dish. The petri dish shall be sealed and the beetles shall be allowed for mating and oviposition.

The culture seeds of pulses shall be examined and screened properly to avoid previous infestation, if any. *Callosobruchus maculatus* shall be maintained at controlled conditions of temperature (27.6±2.5ºC) and relative humidity (70.2±1.03%) in biological oxygen demand incubator. Along with 50 seeds of each pulse in different petri dishes, one pair of newly emerged male and female of *Callosobruchus maculatus* shall also be introduced. Total number of eggs laid by female, hatching, larval- pupal period, total developmental period and number of adults emerged shall be observed carefully. The adult longevity of male and female shall also be noted.

Collection and preparation of plant materials: The study shall be conducted in the Research Laboratory, Department of Biosciences (Division: Zoology), Career Point University, Hamirpur (HP). Different parts of plants (*Leucaena leucocephala*, *Cassia fistula*, *Grewia optiva*, *Carissa carandas* etc.) such as leaves, seeds, roots etc. shall be collected and kept in the laboratory for 7-14 days for air drying under controlled conditions followed by one day sun drying before making powder. After drying, the powder of different parts shall be made separately by an electric grinder in the laboratory and passed through a 60- mesh sieve to get fine powder. Later, powder of different part of plant such as leaf, seed, root etc. shall be kept separately in air tight glass jar and stored at room temperature for experimental use (Hossain *et al.,* 2014).

Screening procedure of plant powders: Screening of botanicals as grain protectants against *C. maculatus* shall be carried out following the primary and secondary screening.

Protocol of primary screening: Fifty grams of healthy seeds of selected pulse shall be weighed by an electric balance, taken in glass containers (300 ml) and mixed properly with powders separately at 20.0 g/kg (w/w) seeds. Five pairs of newly emerged adult beetles of *C. maculatus* obtained from the stock culture shall be sexed and released in each container along with control. Plant materials shall not be used in control treatment. After release of insects, the mouth of the containers shall be tightly closed. This experiment shall be carried out in triplicate. All treated containers shall be kept at temperature (27.6±2.5ºC) and relative humidity (70.2±1.03%) in the BOD incubator for oviposition and development of *C. maculatus*. Dead and alive beetles shall be separated after 7 days from each experimental container and control. The effectiveness of plant materials as protectant against *C. maculatus* shall be assessed. For the determination of oviposition, 100 seeds shall be collected randomly from each container of each treatment and examined under magnifying glass (10x) and compound microscope (Hossain *et al.*, 2014).

The total number of eggs deposited and number of seeds along with eggs (i.e. egg bearing seeds) shall be counted. After each observation, the grains shall be transferred to the respective container for further development. Adults shall be removed and daily growth shall be recorded after emergence. Infested and healthy seeds shall be separated, cleaned,

counted, and finally weighed after completion of adult emergence. Seed damage and weight loss shall also be computed.

Formula:

$$\text{Seed Damage (\%)} = \frac{\text{Initial weight-weight of undamaged seeds}}{\text{Initial weight}} * 100$$

$$\text{Weight loss (\%)} = \frac{UNd - DNu}{U\,(Nd + Nu)} * 100 \qquad \text{(Lal, 1988)}$$

Where, U = Weight of undamaged seeds, D = Weight of damaged seeds,
Nu = Number of undamaged seeds, Nd = Number of damaged seeds.

Protocol of secondary screening: From the primary screening, the most effective powder shall be taken and further tested at lower doses of 10.0, 5.0, 2.5, and 1.25 g/kg seeds. Each dose shall be replicated three times along with control. The screening protocol and observations shall be same as followed in the primary screening (Hossain *et al.*, 2014).

Test on ovicidal and larvicidal effectiveness: To investigate ovicidal and larvicidal properties, 50 seeds along with one day old eggs (ovicidal study) and 1-2 days old larvae (larvicidal study) containing one egg or larva per seed shall be placed in each container and mixed properly with powder of 20.0, 10.0, 5.0, 2.5, and 1.25 g/kg seeds along with control. After proper mixing, the mouth of the containers shall be closed with cap and left undisturbed in the laboratory until adult emergence. The number of adult beetles shall be counted and recorded daily from the first to the last emergence and removed from the containers. After completion of adult emergence, the inhibition shall be computed by using the formula as stated by Shukla *et al.*, (2007).

Formula:

$$\text{Inhibition (\%)} = \frac{(\text{Control mean - Treatment mean})}{\text{Control mean}} * 100$$

Statistical analyses: The collected data shall be tabulated and analyzed using statistical software taking necessary transformations where ever applicable (Gupta, 1986).

Objectives:

1. To study the developmental biology and host range of *Callosobruchus maculatus* on different pulses.
2. To observe the effect of different plant powders on oviposition and adult emergence of *C. maculatus.*
3. To compare the effect of different plant powders on developmental stages of *C. maculatus.*

Thesis Outline:

Chapter 1 (Introduction): It will include general information about the *Callosobruchus macualtus,* morphology, sexual dimorphism, nutritional value of pulses, relationship of *C. maculatus* with pulse etc. It will also include objectives of the study and the research gaps that originated the need for the present study.

Chapter 2 (Literature Review): It will include the deep review of the literature concerning old and latest findings of various researchers regarding the *C. maculatus*, host range, bioinsecticides etc. at national and international level.

Chapter 3 (Materials and Methods): It will deals with methodology of sample collection, maintenance of culture, preparation of plant powders, screening procedure, lab work, lab observations etc.

Chapter 4 (Results and Discussion): In this chapter pest will be described morphologically, data regarding the host range and efficacy of various plant powders shall be presented. Data will be statistically analyzed. There will be the photographs regarding the pest morphology and infestation. It will also include the results of analysis during the study.

Chapter 5 (Summary and Conclusions): This chapter will comprise the conclusion of whole study based on objectives along with summery. The scope of further research will also be discussed.

Research Plan Schedule:

Research Activity	Progress in Months											
	3	6	9	12	15	18	21	24	27	30	33	36
Course work, extensive literature survey or design of work	Aug 2018 to Jul 2019 →											
Research gap identification and development of methodology			Jan 2019 to Dec 2019 →									
Experimental Work and Publications					August 2019 to December 2020 →							
Thesis writing and Publications									Dec 2020 to Jul 2021 →			

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